





Steven M. Ruben  
Appl. No. 10/662,429

Department	_____
Subject	_____
Name	<u>ANN KIM #5</u>
Address	<u>2/4/94 - 5/19/94</u>
	43-648
<b>Computation Notebook</b>	
Dennison Stationery Products Co., Framingham, MA 01701	
	75 Sheets 11 1/2" x 9 1/2" 4x4 Quad.
0 73333-43648 8	

BEST AVAILABLE COPY

Ruben EXHIBIT #88

Department \_\_\_\_\_  
Subject \_\_\_\_\_  
Name ANN KIM #5  
Address 2/4/94 - 5/19/94  
 43-648  
**Computation Notebook**  
Dennison Stationery Products Co., Framingham, MA 01701  
  
75 Sheets  
11" x 9"  
4x4 Quad.  
0 73333 43648 B

Ruben EXHIBIT 2088  
Ruben v. Wiley et al.  
Interference No. 105,077  
RX 2088

HIPANOB Screening 10 7  
Thursday 2/3/94

~~WEEK~~ HIPANOB Screening

HIPANOB

HTP Lib from Scott Messner

$1 \times 10^9$  pfu/ml

12 plates - 7.2 ml LE392  $OD_{600} = 1.0$   
+ 48  $\mu$ l 1:100 dilution

incubate  $37^\circ\text{C}$  5 hrs  
cool  $4^\circ\text{C}$  1 hr.  
do lifts

FRIDAY 2/4/94

Denature  $0.5\text{N NaOH}$ ,  $1.5\text{M NaCl}$

Neutralize  $0.5\text{M Tris pH 8}$ ,  $1.5\text{M NaCl}$

let air dry

Monday 2/7/94

Primmbridge in  $2 \times \text{PIPES buffer}$

$42^\circ\text{C}$  incubator

Fragments - HASC38, HESAY29, HTPANOS - RI/No 13

FRIDAY 2/4/94

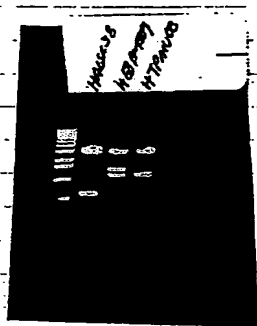
### Fragment Prep:

Digest : HASC38  
HESAY29  
HTPANOS

With EcoRI /XhoI

DNA	10 $\mu$ l
10x #2	5 $\mu$ l
10x BSA	5 $\mu$ l
RI	2
Xho	2
H <sub>2</sub> O	24 $\mu$ l
	40 $\mu$ l

Incubate 37°C, 2 hrs  
Run 1.5  $\mu$ l

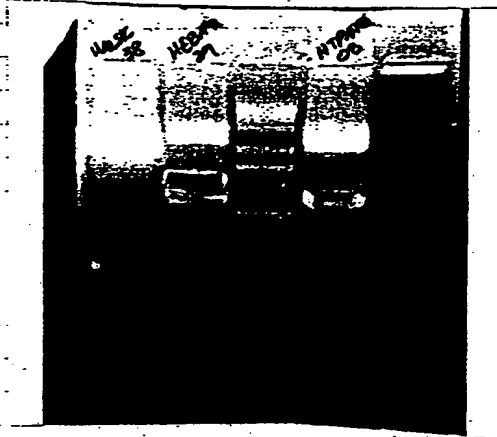


Run on low melt gel + cut out

Monday 2/7/94

Rem on 2<sup>nd</sup> LMPol  
with 1 Kb

Rem 80V 2hrs - 2 1/2 hrs



Cost and bands.

HALSK38	RI/xho	~600 bp.
HEBA129	RI/xho	1.1 Kb
	RI/xho	1.3 Kb
HTPAW03	RI/xho	1.1 Kb

Use ~~trans~~ from Random Prime  
Rymo

# HIV-1 Screening

(pg 8)

25

Tuesday 2/8/94

Pour off Probe

Wash - 0.2XSSC ; 0.1% SDS

Wash 3 x 50°C  
0.2XSSC ; 0.1% SDS  
30min

Put on film  
- 80°C ON.

Wednesday 2/9/94

Develop film.

Total of 37 (+) clones

1 - 4  
2 - 2  
3 - 4  
4 - 3  
5 - 4  
6 - 3  
7 - 4  
8 - 5  
9 - 0  
10 - 0  
11 - 3  
12 - 5

Pick plug into  
600 500 ul SM Buffer  
store 4°C

Do 2° Screening on all (+) clones

plates 1ul → 300ul LE392  
plate onto 100mm plate

HTPAN08 Screening  
2/15/94 Tuesday

lul of phage  $\rightarrow$  300ul LE392  $\Delta$ low = 1.0.

incubate  $37^{\circ}\text{C}$  15 min  
Plate onto NZ4 Plate (100mm)  
in 5ml LB + 0.75% Agarose  
melted  $55^{\circ}\text{C}$

incubate  $37^{\circ}\text{C}$  95 hrs  
Place plate at  $4^{\circ}\text{C}$ .

2/16/94 Wednesday

Do plate lifts  
Denature: 2 min  
Neutralize: 5 min

Phybridge @  $42^{\circ}\text{C}$  in 2x P/PES  
Random Prime fragment

Low Melt. qd fragment 2ul  
5x Buffers 10ul  
34ul

Heat  $100^{\circ}\text{C}$  5 min  
Quench. Chill. Quench again

Add:  
5x dATP Buffer 10ul  
 $\alpha^{32}\text{P}$  dATP 5ul  
Klenow 1ul  
60ul

incubate  $37^{\circ}\text{C}$  15 min

# HPANOS Screening

31

Wednesday 2/16/94

-1.0

nm)  
35

Put through G 25 column

Count 1 ul  
2.23 x 10<sup>6</sup> counts/ul

Use 20ul → 40ul Hyb buffer.  
Boil probe 100°C 5 min in 50ul  
Salmon Sperm DNA - Quick Chill

Membrane 42°C ON

Thursday 2/17/94

Wash 0.2 x SSC; 0.1% SDS

Wash 3 x 55°C  
0.2 x SSC; 0.1% SDS  
30 min / wash

Put on film -80°C

Develop after 3 hrs.

FRIDAY 2/18/94

⊕ Clones

~~1/1~~  
~~2/2~~  
3/3

~~4/4~~  
~~5/5~~  
6/6

~~7/7~~  
~~8/8~~  
9/9

10/10

37 ⊕ clones

1000 clones

On Mem

Recover

	1-3	2-1	3-4	5-2	7-1	8-2	11-1
12-3	1-2	2-2	4-1	5-3	7-2	8-3	11-2
12-4	1-1	3-1	4-2	5-4	7-3	8-4	11-3
12-5	1-4	3-2	4-3	6-2	7-4	6-1	12-1
		3-3	5-1	6-3	8-1	8-5	12-2



HTRANDOB 2<sup>nd</sup> Screening

2/22/94 Tuesday

D<sub>5</sub> min RecusPhage 100  $\mu$ lEXA ~~2~~ 4  $\mu$ lX-1 Blue OD<sub>600</sub> = 1.0 - 200  $\mu$ l

Incubate 37°C 15 min

Add 3 ml LB

Incubate 37°C 2 hrs with aeration

Heat 70°C 20 min

Spin 3K 10 min

Transfer to fresh tubes  
Store at 4°C

2/23/94 Wednesday

5  $\mu$ l  $\rightarrow$  200  $\mu$ l SOLR OD<sub>600</sub> = 1.0

Incubate 37°C 15 min

Plate 100  $\mu$ l onto LB Amp + IPTG/x

Incubate 37°C O/N

DO PCR on Phage mid - (see pg 59)

2/24/94 Thursday

Plates over grown

Re plate -

Add 10 mM HgSO<sub>4</sub> to 1 ml

Incubate 37°C 15 min

Plate 75  $\mu$ l on LB Amp + IPTG/x

Incubate 37°C O/N

(pg 59)

H1rANOS

2° Screening

57

(pg 32)

FRIDAY

2/25/94

Pick clones of each.  
into LBT Amp  
incubate 4 hrs  
Put at 4°C

- 96 Well Dish.  
37°C w aeration

Monday 2/28/94

incubate 37°C 2hrs  
Do PCR.

M13F	0.1
M13R	0.1
Culture	1
H1PAN1575	0.1
10x dNTP	2.1
10x PCR	2.1
Tag	0.15
H <sub>2</sub> O	15.35
	21

use H1PANOS  
Plasmid DNA  
as (+) Control.

PCR Program 69.

Tuesday 3/22/94

Run 10ul on gel with  
1kb ladder

# HIPANOB Screening

pg 32

Wednesday

59  
2/23/14

## PCR Phagemid

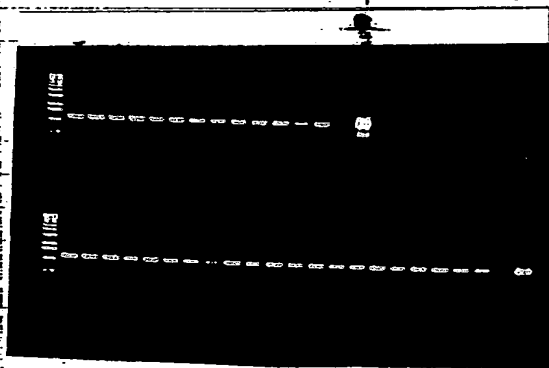
Primer = HIPANOB GGS

Primer	0.1
M13F	0.1
10x PCR	2.1
10x dN	2.1
Phagemid	1
H <sub>2</sub> O	15.45
Taq	0.15
	21.

⊕ Control  
HIPANOB plasmid  
DNA.

## PCR Program #69

Run 10ul + 1Kb ladder.



see pg 32

lecta  
ml  
np

Min

3  
1  
5  
4  
2  
8  
1  
2  
1  
2  
53  
1  
25  
56

~~3/1/94~~ Monday

Pg

(56)

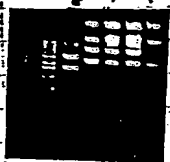
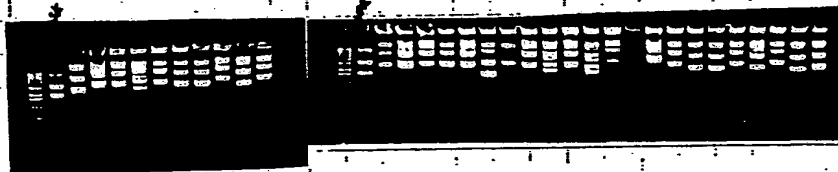
3/1/94 PABed Tuesday

inoculate into 3rd TB + Amp for  
 Boring mini prep  
 incubate 37°C w/aeration

3/2/94 Wednesday

Do boring mini prep

Run Gel on 1% gel w. 10 Kb ladder  
 and pBSK



3/3/94 Thursday

PCR Plasmid DNA

Dilute 1:2000 in H<sub>2</sub>O use 1ul in Rxn

MRF	0.1
HPAN 157-S	0.1
10x dNTP	2.1
10x PCR	2.1
Tag B	0.15
H <sub>2</sub> O	15.45
DNA	1
	21ul

① Control  
+ 100ng  
plasmid

Pg 67

HT...N Screening  
Pg 60

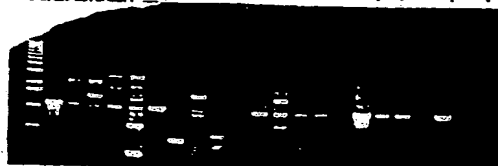
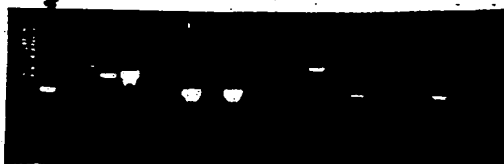
Thursday

67

3/3/94

PCR Program #69.

Run on



• have  
Sequenced  
Reverse

Make internal  
primer

2, 3, 4, 7, 9, 13, 14, 15,  
22, 23, 24, 25, 28, 33

2 S01  
3 S02  
4 S03  
7 S04  
9 S05

13 S06  
14 S07  
15 S08  
22 S09  
23 S10

24 S11  
25 S12  
28 S13  
33 S14

HTPAN 03, 04, 13 & 14 look good.

Sequence, with gene specific Primers  
3' → 5'

Forward Sequence did not match.

Re screen

68

## HTPANOS SCREENING

4/19/94 Tuesday

Plate 20 plates  
~40,000 plaques / plate $1 \times 10^7$  pfs/ml.0.8 ul phage into 6 ml LE392  $OD_{600} =$ Incubate  $37^\circ\text{C}$  15 min

Put into melted LB + Agarose (0.75%)

Plate onto 150 mm NZY plates

Incubate  $37^\circ\text{C}$  5-6 hrsPut at  $4^\circ\text{C}$  O/N.

4/20/94 Wednesday

Denature 2 min

Neutralize 5 min

Pb Prehybridize 2 hr  $-42^\circ\text{C}$   
at 2x PIPESRandom Prime  
HTPANOS xho / RI

DNA	1 ul
5x Primers	10 ul
H <sub>2</sub> O	23 ul
	<u>34</u>

Heat  $100^\circ\text{C}$  5 min

Quick Chill Spin

Chill on ice

Add	10 ul	5x Buffer	dATP
	5 ul	$\gamma\text{-P}$ dATP	
	1 ul	Klenow	

MPANOS

Screening

69

Wednesday 9/20/94

Incubate  $37^{\circ}\text{C}$  10 min

Put through G-25 column

Count  $\text{ml}$ .

$\sim 1.3 \times 10^4$  30  $\text{ml}$ .

Boil 5 min

Chill on ice

Add 20  $\mu\text{PIES}$  H<sub>2</sub>O

Incubate  $42^{\circ}\text{C}$  O/N

Thursday 9/21/94

Wash 0.2XSSC, 0.1% SDS.

Wash 3X at  $65^{\circ}\text{C}$   
0.2XSSC, 0.1% SDS.

Put on film  $-80^{\circ}\text{C}$   
Signal not very strong - so  
leave until Monday

Monday 9/25/94

Develop film

H<sub>2</sub>P<sup>+</sup> 24  
H<sub>2</sub>N 13

70

TRAN 08

4/25/94 Monday

Pick plugs into 300ul of  
3m.

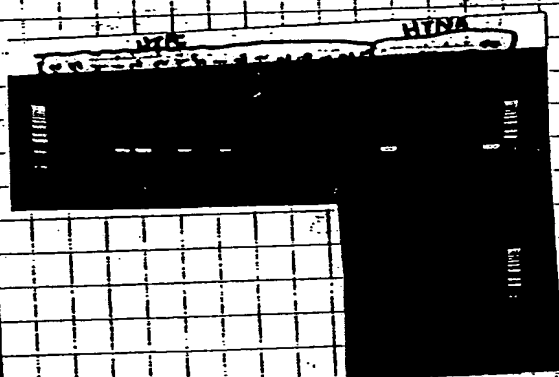
Incubate at Room 16°C ON.

4/26/94 Tuesday

PCR Reaction Plaque

M13F	0.2
157-S	0.2
10X dN	2.5
10X PCR	2.5
H <sub>2</sub> O	14.4
Taq	0.2
Plaque	5
	25

Program # 58

Do 2<sup>nd</sup> screens of ⊕ clones

PA  
PA



# HTPAN 08

129

(pg 70)

Wednesday

4/27/94

Use small NZY plates -  
 \* No plates so had to make own  
 NZY Plates \*

Thursday

4/28/94

LE392.0000 = 0.5 - 100  $\mu$ l  
 + 1.500 Dilution of phage.

Incubate 37°C 6 hrs  
 Cool at 4°C ON

FRIDAY

4/29/94

Do plate lifts  
 place orientation marks.

Monday

4/5/94

Denature 2min 0.5N NaOH / 1.5M NaCl  
 Neutralize 5min 0.5M Tris pH 8.0 / 1.5M NaCl  
 Wet on 2x SSC 5min.

Allow filters to dry

133

## H7PANOB

5/3/94

Tuesday

b

Probe Blots

80°C 1 hour

make probe

H7PANOB xhs /RI

DNA	1ul
5x PRIMERS	10ul
H <sub>2</sub> O	23ul
	<hr/> 34ul

Heat 100°C 5 min

Quick Spin

Place on ice

Add

5x dATP Buffer	10ul
<sup>32</sup> P dATP	5ul
Isenon	1ul
	<hr/> 5ul

Incubate 37°C 10 min

put through Gels 6-25 column

Count 1ul

1.04 x 10<sup>6</sup> count /ul ~ 50ul~~Use 200~~

Use 30ul Hyb Buffer

Prehybridize Blots	1 hr in
in 8 PIPES	buffer at 42°C

Pour off Prehyb add the Hyb buffer

Tuesday

5/3/94

Heat

Probe + 50ul Salmon Sperm DNA  
at 100°C 5min

Quick Chill

Add 70ul to Hyb buffer w/ lifts  
Incubate 42°C O/N with  
Slight Agitation.

Wednesday 5/4/94

Wash Blots -

Rinse 1x 0.2xSSC / 0.1% SDS

Wash 3x in

0.2xSSC + 0.1% SDS  
at 65°C

No Film Cassettes

So store at RT in 0.2xSSC + 0.1% SDS

Thursday

5/5/94

Put on film

-80°C for 1 hrs

Develop film.

Picked ③ clones

H. PANOS

5/6/94

Thurs. FEIDAY

Have full length

found that 504, 513, 514

fit into a contig &amp;

When Blasted against HGS/TIGR  
Database returned a clone

which when blasted against

HGS/TIGR EST Produced a match  
to HTPAN.

Sequence to get full length

5/8/94

Make Oligos to sequence

More 3' - forward Forward end

5/10/94

Monday

Submit for sequencing

5/11/94

Tuesday

Transform Df/50 w/o - prason  
& Do maxi Plasm.

5/12-5/13 off

- Carrie took plasmid out of  
37°C. to 4°C

(pg 136)

HTPAN 08

(10/10/132)

5/11/99 Monday

Pick &amp; Clous of lawn

SO<sub>4</sub>, S<sub>13</sub> & S<sub>14</sub> into LB Amp  
in 1/6 well dish

Incubate 37°C ON

5/17/99 Tuesday

Run PCR using M13 F &amp; Rev.

		25X
M13 F & M13 R	0.1	25
10x dNTP	2	50
10x PCR	2	50
H <sub>2</sub> O	13.7	342.5
Topo	0.2	50
Antibio	2	—

Run PCR Program #69

95°C 5min

95°C 20sec

55°C 20sec

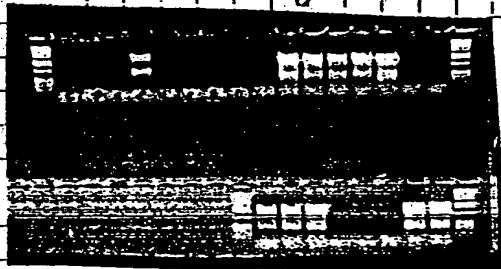
72°C 1min

72°C 7.5min

4°C

30x

Run 10ul on gel with 1kb ladder



Tuesday 5/17/94

Inoculate 400 ml  
TB + Amp with

S04 - 3

S13 - 5

Incubate 37°C w/ aeration

Wednesday 5/18/94

Main Prep

Spin Culture 5K 10 min

Pour off

Resuspend in 15 ml Resuspension  
Buffer

Add 75 ml Cell Lysis Buffer

Add 15 ml Neutralization Buffer

Spin 8K 10 min

Transfer to fresh Tube

Add Isopropanol to 0.6 volumes

Mix

Spin 9K 30 min

Pour off

Resuspend pellet in 1 ml TE

Transfer to 2 - 1.5 ml eppendorf

- 2x Phenol / SEVAG

- 2x SEVAG

Ethanol ppt 1/10 vol 3M NaAc

2x vol Ethanol

Inc 15 min

Spin 15 min

Pour off supernatant

2x 70% Ethanol wash

1x 100% Ethanol wash

Dry pellet & Resuspend

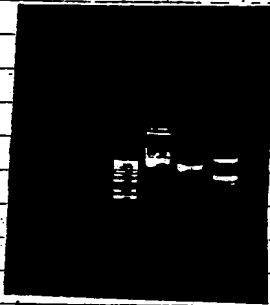
Read OD<sub>260</sub>/OD<sub>280</sub>

Run 2 ul on gel with markers

138

HTPAN08

5/18/94 Wednesday

5/19/94 Thursday  
Read O.D.<sub>260</sub> / 280

1.100 Dilution

	abs 260.0 nm	abs 280.0 nm	big abs 320.0 nm	260.0 nm 280.0 nm	280.0 nm 260.0 nm
HTPAN08	0.0011	0.0007	0.0005	4.0362	0.2478
HTPAN08S04	0.0679	0.0344	-0.0036	1.8790	0.5322
HTPAN08S13	0.1344	0.0674	-0.0049	1.9259	0.5192
	0.6327	0.3200	-0.0027	1.9689	0.5079

0.34 ug/lul.  
0.6 ug/lul.  
3.16 ug/lul.

Submit for Sequencing

HTPAN08

Fc

Rc

R157S

RP01b

R318S

RP03b

R166S

RP05b

R338S

RP06b

F488A

RP07b

HTPAN08S04

Fc

Rc

RP01

RP03b

RP05

RP06

RP07

HTPAN08S04 pol RP10

RP10

HTPAN08S13

Fc

Rc

RP01

RP03b

RP05

RP06

RP07

HTPAN08S13 pol RP09

RP09

RP10

Pg 53 Book #168  
AMC #6

Sample ID	260.0 nm		280.0 nm		260.0 nm		280.0 nm	
	abs	abs	bkg abs	abs	abs	abs	abs	abs
1mer 1HES621	0.0558	0.0297	-0.0001	1.8768	0.5328	0.46ug/ml	73.4 pmol/l	
1mer 2HPR480	0.0508	0.0336	-0.0005	1.5008	0.6683	0.42ug/ml	67 pmol/l	

2/2/94

2/7/94

Sample ID	260.0 nm		280.0 nm		260.0 nm		280.0 nm	
	abs	abs	bkg abs	abs	abs	abs	abs	abs
1HTAN08F 3185	0.0473	0.0364	0.0044	1.2674	0.7890	0.39ug/ml	59.1 pmol/l	
2HTAN08R 4050	0.0350	0.0176	0.0054	1.7582	0.5687	0.29ug/ml	46.1 pmol/l	
3HTAN08R 665	0.0499	0.0270	0.0009	1.8805	0.5318	0.41ug/ml	65.7 pmol/l	

2/11/94

Sample ID	260.0 nm		280.0 nm		260.0 nm		280.0 nm	
	abs	abs	bkg abs	abs	abs	abs	abs	abs
1mer 1HMAA 61-2055	0.0430	0.0324	0.0065	1.4044	0.7121	0.22ug/ml	37 pmol/l	
1mer 2HMTN 17-2595	0.0384	0.0237	-0.0000	1.6219	0.6166	0.19ug/ml	30 pmol/l	

2/15/94

Sample ID	260.0 nm		280.0 nm		260.0 nm		280.0 nm	
	abs	abs	bkg abs	abs	abs	abs	abs	abs
1 5512	0.0300	0.0194	0.0023	1.6231	0.6161			
2 5513	0.0324	0.0164	0.0011	2.0444	0.4892			

3/4/94

Sample ID	260.0 nm		280.0 nm		260.0 nm		280.0 nm	
	abs	abs	bkg abs	abs	abs	abs	abs	abs
1HBE64 95	0.0551	0.0346	0.0015	1.6215	0.6167	0.3ug/ml	50 pmol/l	
2HBE68 97	0.0586	0.0286	0.0021	2.0539	0.4889	0.3ug/ml	51.6 pmol/l	



150

3/8/94

Sample ID	abs		bkg abs	260.0 nm	280.0 nm
	260.0 nm	280.0 nm		280.0 nm	260.0 nm
1153					
25677 HASK POI	0.0691	0.0450	-0.0006	1.5287	0.6541
35676 HTPANOS	0.0935	0.0524	0.0017	1.8117	0.552089 pmol/ul
45675 HESAY POI	0.1129	0.0588	0.0023	1.9576	0.5108 102 pmol/ul
55678 HESAY POI	0.0750	0.0528	0.0022	1.4427	0.6931 79 pmol/ul
55678 HESAY POI	0.0827	0.0505	0.0027	1.6746	0.5972 89 pmol/ul

3/10/94

Sample ID	abs		bkg abs	260.0 nm	280.0 nm
	260.0 nm	280.0 nm		280.0 nm	260.0 nm
5697					
1HE9MF3 142	0.0857	0.0477	0.0010	1.8121	0.5519 81 pmol/ul
2HE7SE29 234	0.0604	0.0385	-0.0009	1.5565	0.6425 60 pmol/ul
5688					
3HTPANOS 485	0.0536	0.0283	-0.0017	1.8443	0.5422 51 pmol/ul
5685					
4 HPLBTS2 222	0.0551	0.0375	-0.0010	1.4573	0.6882 55 pmol/ul
5686					
5 HPRAL36 554	0.0268	0.0179	-0.0000	1.4972	0.6679 30 pmol/ul

3/10/94

Sample ID	abs		bkg abs	260.0 nm	280.0 nm
	260.0 nm	280.0 nm		280.0 nm	260.0 nm
5756					
1HTPAUCS 1244	0.0467	0.0270	0.0018	1.7815	0.5613 61 pmol/ul

3/17/94

Sample ID	abs		bkg abs	260.0 nm	280.0 nm
	260.0 nm	280.0 nm		280.0 nm	260.0 nm
5780					
1740186	0.0746	0.0417	-0.0003	1.7828	0.5609 19mer 70 pmol/ul
5781					
2740187	0.0770	0.0533	-0.0005	1.3833	0.7228 19mer 81 pmol/ul
5782					
3740180	0.0762	0.0508	-0.0021	1.4809	0.6752 19mer 80 pmol/ul
5783					
4740184	0.0847	0.0564	-0.0024	1.4806	0.6754 21mer 80 pmol/ul
5784					
6740183	0.0463	0.0310	-0.0028	1.4579	0.6859 20mer 46 pmol/ul
5785					
7740137	0.0732	0.0437	-0.0028	1.6350	0.6116 19mer 77 pmol/ul
5786					
8740137	0.0658	0.0527	-0.0019	1.2388	0.8073 19mer 69 pmol/ul

3/18/94

Sample ID	abs		bkg abs	260.0 nm	280.0 nm
	260.0 nm	280.0 nm		280.0 nm	260.0 nm
5787					
1	-0.0007	-0.0016	0.0005	0.6323	1.8786
5788					
2740140RPO10	0.0538	0.0362	-0.0022	1.4574	0.6882 59 pmol/ul
5799					
3740144RPO10	0.0137	0.0056	-0.0021	2.0592	0.4856 15.2
5800					
4740147RPO10	0.0111	0.0045	-0.0031	1.8718	0.5343 11.1 pmol/ul
5809					
5HETAS6 R PO10	0.0061	0.0020	-0.0034	1.7501	0.5714 64 pmol/ul
5810					
6HETAS6 R PO10	0.0377	0.0129	-0.0036	2.5057	0.3991 39 pmol/ul
5811					
7 HAFBOS R PO10	0.0244	0.0129	-0.0043	1.6674	0.5998 24.4 pmol/ul

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